Genome wide association study of apparent treatment resistant hypertension in the CHARGE consortium: The CHARGE Pharmacogenetics Working Group

Running head: GWAS of aTRH

Word counts
Abstract: 249
Text: 2003
Tables: 1
References: 20

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Keywords: treatment resistant hypertension, genome wide association study, severe hypertension

ABSTRACT

Background: Only a handful of genetic discovery efforts in apparent treatment resistant hypertension (aTRH) have been described. Methods: We conducted a case-control genome-wide association study (GWAS) of aTRH among persons treated for hypertension, using data from 10 cohorts of European ancestry (EA) and 5 cohorts of African ancestry (AA). Cases were treated with 3 different antihypertensive medication classes and had blood pressure (BP) above goal (systolic (SBP)≥140 mm Hg and/or diastolic (DBP)≥90 mm Hg) or 4 or more medication classes regardless of BP control (n_{EA} =931, n_{AA}= 228). Both a normotensive control group and a treatment-responsive control group were considered in separate analyses. Normotensive controls were untreated (n_{EA} = 14210, n_{AA}= 2480) and had SBP/DBP <140/90 mm Hg. Treatment-responsive controls (n_{EA} = 5266, n_{AA}= 1817) had BP at goal (<140/90 mm Hg) while treated with one antihypertensive medication class. Individual cohorts used logistic regression with adjustment for age, sex, study site and principal components for ancestry to examine the association of SNPs with case-control status. Inverse variance-weighted fixed-effects meta-analyses were carried out

using METAL. Results: The known hypertension locus, *CASZ1*, was a top finding among EAs ($P=1.1*10^{-8}$) and in the race-combined analysis ($P=1.5*10^{-9}$) using the normotensive control group (rs12046278 OR=0.71[95% CI 0.6-0.8]). SNPs in this locus were robustly replicated in the Million Veterans Program (MVP) study in consideration of a treatment-responsive control group. There were no statistically significant findings for the discovery analyses including treatment-responsive controls. Conclusion: This genomic discovery effort for aTRH identified *CASZ1* as an aTRH risk locus.

INTRODUCTION

Apparent treatment-resistant hypertension (aTRH) is an extreme form of hypertension (HTN) characterized by the use of 4 or more antihypertensive (AHT) medication classes to achieve blood pressure (BP) control. The estimated prevalence of aTRH in population-based studies is between 12-15% among adults with hypertension and higher among clinic-based populations, e.g. >25% in those with chronic kidney disease (CKD).[1, 2] Risk factors for aTRH are increasing age, obesity, reduced kidney function and African-American race.[1] Research shows that individuals with aTRH are at an increased risk for cardiovascular disease (CVD) events when compared to individuals with controlled HTN, demonstrating a need to understand the cause of nonresponse in order to improve BP control.[3] We hypothesized that identifying the genetic architecture may shed light on distinct underlying patho-biology.

Published genetic studies of aTRH have reported limited findings and are lacking in comparison to hypertension. [4-7] The current study comprises European ancestry (EA) and African

ancestry (AA) studies from the *Cohorts for Heart and Aging Research in Genomic Epidemiology* (CHARGE) consortium, for a case-control study of aTRH that capitalizes on epidemiological data characterized by deep phenotyping. Common genetic variants in 931 EA aTRH cases were compared to 14210 normotensive controls and separately to 5266 treatment-responsive controls, while 228 AA aTRH cases were compared to 2480 normotensive controls and separately to 1817 treatment-responsive controls. Results were replicated in an aTRH case-control dataset from the Million Veterans Program (MVP).

METHODS

Ten studies contributed data on EA participants while 5 studies contributed data on AA participants (Supplemental File Section 1). Data on medication use were extracted by medication inventory, self-report, or computerized databases once for cohorts with cross-sectional data, or at each BP measurement for those with longitudinal data (Supplementary File Section 1). AHT medications counted towards the sum of classes are described in Supplemental Table 1. Combination products were therapeutically co-classified based on their active ingredients. All diuretics were counted as one class including potassium sparing diuretics.

Participants with conditions that may lead to secondary forms of hypertension (including eGFR <30 mL/min/1.73m^2 or BMI >40 kg/m^2) were excluded. aTRH cases were defined as those treated with 3 AHT medication classes and BP above goal (systolic BP (SBP)≥140 mm Hg and/or diastolic BP (DBP)≥90 mm Hg) or 4 or more AHT medication classes regardless of BP control. aTRH cases fitting the above definition who were not treated by a diuretic were excluded.[8] The analysis included two control groups: 1. *Normotensive controls*: participants not hypertensive and not treated with an AHT medication; and 2. *Treatment-responsive controls*: participants who had BP at goal (<140/90 mm Hg) on treatment with one AHT medication class. Details of the case

and control definition in cohorts with longitudinal data are described in Supplementary File Section 1.

Genome-wide single nucleotide polymorphism (SNP) genotyping was performed within each study using commercial genotyping arrays (Supplementary File Table 2). Cohorts most commonly imputed to the 1000 genomes version 3 reference panel. After imputation cohorts filtered out SNPs with imputation quality score <0.3. SNPs with MAF <5% and which were not represented in two or more cohorts were filtered out at the meta-analysis stage.

Statistical Analysis

Logistic regression models or generalized estimating equations were used for case-control association analysis (Supplementary File Table 3). The variable of interest was SNP dosage of the effect allele. Models were adjusted for age, sex and study-specific covariates (e.g., study site, principal components for ancestry and, if applicable, exchangeable correlation matrices to account for family relatedness). For cohorts with longitudinal data, the average age across the visits included was used as the covariate. In total, there were four models, one for each control group and one for each ancestry grouping. Inverse variance-weighted, fixed-effects meta-analysis was performed for each of the four strata, using METAL software (www.sph.umich.edu/csg/abecasis/metal/). Statistical heterogeneity across studies was evaluated using Cochran's χ^2 test (Q-test). P-values $<5\times10^{-8}$ indicated genome-wide significant results. Results of the race-stratified analyses from METAL were then combined using a similar approach (one meta-analysis per control group). Linkage disequilibrium (LD) was evaluated using the rAggr tool (http://raggr.usc.edu/). Regional plots were created using Locus Zoom with a window of 500kb (v0.4.8).[9] In a sensitivity analysis of top SNP results we conducted a meta-analysis that included only cohorts with >50 cases.

Replication

We sought replication in non-Hispanic EA (78%) and AA (22%) MVP participants (Supplemental File Section 1). [10, 11] Participants with eGFR ≥60 ml/min/1.73m^2 were included. Total numbers of samples across ethnicities included 16,833 cases (11762 EAs and 5071 AAs) and 53,931 controls (42850 EAs and 11081 AAs). Cases were defined using the same definition as the discovery analysis. Controls were patients who achieved BP control (<140/90 mmHg) on one or two medication classes. Case-control status was regressed onto additively coded genotypes imputed to 1000 Genomes phase 3 version 5, adjusting for age, age^2, sex, body mass index, and 10 principal components within ethnicity using SNPTEST v2.54. Genotyping, quality control (QC), and imputation procedures have been described [10].

RESULTS

Overall EA and AA cases were older than controls and more likely male (Supplemental Tables 4a and 4b). The average number of AHT medication classes for EA cases ranged from 3.2 to 3.8 and from 3.3 to 3.9 for AAs. Across the individual cohort GWAS analyses, there was not excessive evidence for the deviation of P-values from their expected values (Supplementary File Table 5). Manhattan plots and QQ plots for each discovery meta-analysis are presented in Supplemental Figures 1a-d and 2a-d for the comparison of AA cases to AA normotensive controls, EA cases to EA normotensive controls, AA cases to AA treatment-responsive controls and EA cases to EA treatment-responsive controls, respectively. Meta-analysis corrected inflation that existed in the cohort-specific analyses.

The top 5 results for each case-control model are presented in Table 1. When comparing aTRH cases to normotensive controls, the top finding for AAs was rs76967376 intronic to myosin-

Vb (*MYO5B*). At that SNP, the direction of effect was consistent across each of the 5 cohorts and the odds of being a case were 2.65 [95% CI 1.9-3.8] times higher among those with the A allele versus the C allele. Among EAs, the top findings for the normotensive control comparison were intronic to castor zinc finger 1 (*CASZ1*). In the race-combined analysis *CASZ1* rs12046278 T carriers were less likely to be a case ($P=1.5*10^{-9}$, OR=0.71[95% CI 0.63-0.80]). Another SNP within 3500 bp to DNA (cytosine-5-)-methyltransferase 3 alpha *(DNMT3A)* was associated with aTRH ($P=4.9*10^{-8}$) in the race-combined analysis using normotensive controls. Regional plots (Supplemental Figures 3-5) for rs76967376 (*MYO5B*), rs12046278 (*CASZ1*) and rs11674660 (near *DNMT3A*) display linkage disequilibrium support for these top findings. Results of the race-combined analysis are presented in Supplemental File Table 6 and Supplemental Figure 6.

When comparing aTRH cases to treatment-responsive controls no SNP was statistically significant after correcting for multiple testing in either racial strata. Race-combined analysis did not increase the significance of top hits. In the sensitivity analysis limiting contributing cohorts to those with >50 cases results were generally consistent with the main findings in Table 1 (Supplemental Table 7).

The MVP cases in the replication study were older (63±9 vs 62±10 years for EAs and 58±9 vs. 56±10 years for AAs) and had slightly higher BMI compared to the treatment-responsive controls. Results for AAs as well as the EAs for the treatment-responsive control group were not replicated in the MVP. However, results from the EA discovery for the normotensive control group were robustly replicated with the same direction of effect for SNPs in *CASZ1* ($P<5*10^{-8}$) and the direction of association for rs11674660 intergenic to *DNMT3A, DTNB* was consistent in direction but not statistically significant ($P=0.09$) (Supplemental Table 8).

CONCLUSIONS

While the genetics of BP and essential hypertension have been extensively investigated, few genetic studies have explored genes associated with less common and more severe aTRH. Using data available from observational epidemiological cohort studies, the current meta-GWAS study examined SNPs associated with aTRH in EA and AA cases with respect to two different control sets. Our study confirmed the known BP locus, *CASZ1,* as being robustly associated with aTRH in the discovery and replication dataset. Other notable findings, *MYO5B* and *DMNT3A/DTNB,* warrant additional replication efforts.

To our knowledge our top finding in the AA strata (rs76967376 in *MYO5B* involved in cell trafficking and plasma membrane recycling) has been associated with lipid levels in previous GWAS, but has not been associated with hypertension. The nearest published BP locus (rs745821) is in the MAK4 gene (~505kb in distance) and is not in LD with our finding (r^2 <0.01).[12] At least one animal model has reported *MYO5B* may regulate an atrial voltage-gated potassium channel (Kv1.5) important for cardiac excitability.[13] This result was not replicated in the MVP aTRH case-control dataset. Future studies may still be warranted given the differences in the replication dataset that used treatment-responsive controls with eGFR ≥60 ml/min/1.73m^2. The top finding among EAs was the known hypertension locus *CASZ1*, a zinc finger transcription factor which plays a key role in cardiac development and postnatal adaptation.[14] The gene has been previously associated with BP and hypertension in Asian ancestry and EA populations.[15-17] The biological role of *CASZ1* in aTRH needs additional investigation but may be related to expression changes in genes that regulate blood pressure or AHT response.[18] Taken together the significant results from the discovery and replication analysis suggest *CASZ1* is an aTRH locus among EAs. The result for the top SNP was consistent but marginally significant for AAs in CHARGE (OR=0.69[95% CI 0.48-0.99];P=0.04 for the T allele). Rs880315 in *CASZ1* from Table 1 was marginally

significant in MVP blacks (OR=1.09[95% CI 1.03-1.15];P=0.008 for the C allele). Loci near *DMNT3A/DTNB* on chromosome 2 have been identified in a recent BP GWAS study (~300 kb downstream of *ADCY3*) though rs11674660 from our study and previously published *ADCY3* rs55701159 are not in LD (r^2<0.01).[12] *DMNT3A* is causal for clonal hematopoiesis of indeterminate potential (CHIP), and mutations in *DMNT3A* have been associated with coronary heart disease.[19] The isoprenylcysteine carboxyl methyltransferase (*ICMT*) locus was the only gene near a previously identified HTN gene (~15kb downstream of *RNF207 rs709209*) [20] that we report on for the treatment-responsive control group. The SNP rs11674660 near *DMNT3A/DTNB* and rs146183009 in *ICMT* were not replicated in the MVP.

We also compared our results with published GWAS studies.[5, 6] In the electronic MEdical Records & GEnomics study among 3006 cases and 876 treatment-responsive controls there were no statistically significant findings. In the INternational VErapamil SR Trandolapril STudy GENEtic Substudy, a SNP (rs12817819) in ATPase Plasma Membrane Ca2+ Transporting 1 (*ATP2B1*) was associated with aTRH in EAs and Hispanics. In our data, SNPs in *ATP2B1* were most strongly associated with aTRH when cases were compared to normotensive controls [AAs rs58302337 (*P*=0.001), rs12580678 (*P*=0.004); EAs rs1401982 (*P*=0.006)] versus treatment responsive controls [AAs rs152754 (*P*=0.01); EAs rs34205054 (*P*=0.006)]. Differences between these studies and our own include the use of clinical rather than observational populations and the consideration of only controlled hypertensive patients as controls.

Strengths of the current study include collaboration among well-characterized CVD cohorts for which BP measurement and the recording of AHT information was a focus. Further, we replicated our findings in a large dataset with comparable ethnic groups. However, aTRH is complex and our study had several weaknesses including lack of information on white coat

hypertension, adherence information, and medication dosage data which may contribute to phenotypic misclassification which could dilute our results. We were unable to distinguish AHT use for conditions other than hypertension such as glaucoma. Other limitations included heterogeneity among study populations regarding phenotypic focus (e.g. obesity and CVD) and different methods for the measurement of BP. Finally, the case-control group available for the replication analysis was not identical to our discovery dataset.

Despite being common among persons with hypertension, little is known about the genetic etiology of aTRH. In this discovery and replication effort the main finding included a transcription factor and known hypertension locus involved in cardiac development (*CASZ1*). *MYO5B* and *DMNT3A/DTNB* were biologically interesting cardiovascular candidates that were not replicated but remain worthy of further investigation for this severe form of hypertension.

ACKNOWLEDGEMENTS and FUNDING

Age, Gene, Environment, Susceptibility—Reykjavik Study (AGES): This study has been funded by NIH contracts N01-AG-1-2100 and HHSN271201200022C, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The study is approved by the Icelandic National Bioethics Committee, VSN: 00-476 063. The researchers are indebted to the participants for their willingness to participate in the study.

Atherosclerosis Risk in Communities Study (ARIC): The ARIC study has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services (contract numbers HHSN268201700001I, HHSN268201700002I, HHSN268201700003I, HHSN268201700004I and HHSN268201700005I), R01HL087641, R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

Cardiovascular Health Study (CHS): This research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, HHSN268200960009C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083 and N01HC85086; and NHLBI Grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, U01HL130114, and R01HL085251 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI Grant UL1TR000124 and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) Grant DK063491 to the Southern California Diabetes Endocrinology Research Center. JSF was supported by K08HL116640.

Heart and Vascular Health Study (HVH): The Heart and Vascular Health Studies has been funded in part by NHLBI grants R01HL085251 and R01HL073410.

HyperGEN, Genetics of Left Ventricular Hypertrophy (HyperGEN): The HyperGEN: Genetics of Left Ventricular Hypertrophy is ancillary to the Family Blood Pressure Program, http://clinicaltrials.gov/ct/show/NCT00005267. Funding sources included National Heart, Lung, and Blood Institute grant R01HL055673 and cooperative agreements (U01) with the National Heart, Lung, and Blood Institute: U01HL054471, U01HL54515 (UT); U01HL054472, 471 U01HL054496 (MN); U01HL054473 (DCC); U01HL054495 (AL); U01HL054509 (NC).

Netherlands Epidemiology of Obesity Study (NEO): The authors of the NEO study thank all individuals who participated in the Netherlands Epidemiology in Obesity study, all participating general practitioners for inviting eligible participants and all research nurses for collection of the data. We thank the NEO study group, Pat van Beelen, Petra Noordijk and Ingeborg de Jonge for the coordination, lab and data management of the NEO study. The genotyping in the NEO study was supported by the Centre National de Génotypage (Paris, France), headed by Jean-Francois Deleuze. The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area Vascular and Regenerative Medicine. Dennis Mook-Kanamori is supported by Dutch Science Organization (ZonMW-VENI Grant 916.14.023).

The Jackson Heart Study (JHS): is supported and conducted in collaboration with Jackson State University (HHSN268201800013I), Tougaloo College (HHSN268201800014I), the Mississippi State Department of Health (HHSN268201800015I) and the University of Mississippi Medical Center (HHSN268201800010I, HHSN268201800011I and HHSN268201800012I) contracts from the National Heart, Lung, and Blood Institute (NHLBI) and the National Institute for Minority Health and Health Disparities (NIMHD). The authors also wish to thank the staffs and participants of the JHS. LMR is funded by T32 HL12998.
The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

Multi-ethnic Study of Atherosclerosis (MESA): MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-000040, UL1-TR-001079, UL1-TR-001420. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Genotyping was performed at Affymetrix (Santa Clara, California, USA) and the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0.. Also supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR001881, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

The Prospective study of Pravastatin in the elderly at risk (PROSPER): The PROSPER study was supported by an investigator initiated grant obtained from Bristol-Myers Squibb. Prof. Dr. J. W. Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). Support for genotyping was provided by the seventh framework program of the European

commission (grant 223004) and by the Netherlands Genomics Initiative (Netherlands Consortium for Healthy Aging grant 050-060-810).

AfterEU: The AfterEU study is the Danish part of the EURAGEDIC study which was supported by the European Commission (contract QLG2-CT-2001–01669). The genotyping for this study was part of the Genetics of Diabetic Nephropathy (Gen DN) study, primarily funded by Juvenile Diabetes Research Foundation (JDRF) International Prime Award Number 17-2013-8. TSA was also funded by the GenDN grant and acknowledges the same. Additionally, TSA was supported by internal funding from Steno Diabetes Center Copenhagen, Gentofte, Denmark and from the Novo Nordisk Foundation (Steno Collaborative 2018) Grant NNF18OC0052457 and acknowledges the same. No potential conflicts of interest relevant to this article were reported. The authors acknowledge the technical assistance of Tina R. Juhl, Anne G. Lundgaard, Berit R. Jensen, and Ulla M. Smidt. In addition, the authors thank Dr. Steve Rich, who was involved in genotyping from the University of Virginia, USA.

This research is based on data from the Million Veteran Program, Office of Research and Development, Veterans Health Administration, and was supported by award I01BX003360 to Adriana M. Hung. This work was supported using resources and facilities of the VA Informatics and Computing Infrastructure (VINCI), VA HSR RES 13-457. Jacklyn N. Hellwege and Ayush Giri are supported by the Building Interdisciplinary Research Careers in Women's Health career development program grant K12HD043483 (PI: K.E. Hartmann). Todd L. Edwards was supported by NIH/NHLBI grant HL121429.

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- Philadelphia VA Medical Center (Darshana Jhala)
- Phoenix VA Health Care System (Samuel Aguayo)
- Portland VA Medical Center (David Cohen)
- Providence VA Medical Center (Satish Sharma)
- Richard Roudebush VA Medical Center (John Callaghan)
- Salem VA Medical Center (Kris Ann Oursler)
- San Francisco VA Health Care System (Mary Whooley)
- South Texas Veterans Health Care System (Sunil Ahuja)
- Southeast Louisiana Veterans Health Care System (Amparo Gutierrez)
- Southern Arizona VA Health Care System (Ronald Schifman)
- Sioux Falls VA Health Care System (Jennifer Greco)
- St. Louis VA Health Care System (Michael Rauchman)
- Syracuse VA Medical Center (Richard Servatius)
- VA Eastern Kansas Health Care System (Mary Oehlert)
- VA Greater Los Angeles Health Care System (Agnes Wallbom)
- VA Loma Linda Healthcare System (Ronald Fernando)
- VA Long Beach Healthcare System (Timothy Morgan)
- VA Maine Healthcare System (Todd Stapley)
- VA New York Harbor Healthcare System (Scott Sherman)
- VA Pacific Islands Health Care System (Gwenevere Anderson)
- VA Palo Alto Health Care System (Philip Tsao)
- VA Pittsburgh Health Care System (Elif Sonel)
- VA Puget Sound Health Care System (Edward Boyko)
- VA Salt Lake City Health Care System (Laurence Meyer)
- VA San Diego Healthcare System (Samir Gupta)
- VA Southern Nevada Healthcare System (Joseph Fayad)
- VA Tennessee Valley Healthcare System (Adriana Hung)
- Washington DC VA Medical Center (Jack Lichy)
- W.G. (Bill) Hefner VA Medical Center (Robin Hurley)
- White River Junction VA Medical Center (Brooks Robey)
- William S. Middleton Memorial Veterans Hospital (Robert Striker)

DISCLOSURE: No conclict of interest to declare for MRI

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